香港大學
The University of Hong Kong

GEM
The University of Hong Kong 2016

DNA nanostructures for disease diagnosis through miRNA-induced structural transformation

透過微核糖核酸誘發之結構轉變來診斷疾病的去氧核醣核酸納米結構

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Introduction

About iGEM

The International Genetically Engineered Machine (iGEM) competition is one of the largest annual world-class prestigious research competitions. Each year, participating teams engage in a research project, which they would then present at the Giant Jamboree in Boston, USA during the fall. The teams' performances would be judged by professionals for a number of awards, such as Best Project Awards and Best Presentation Awards.

Apart from the research aspect, iGEM teams also have to promote synthetic biology in their own region. They would use all kinds of pathways, including public exhibitions, laboratory demonstrations, interviews and others to raise public awareness of synthetic biology. They hope to arouse interest towards the field as well as allow the public to understand the potential issues behind the new technologies.

And this, is exactly what we are doing now.

簡介

國際基因工程機械比賽

國際基因工程機械比賽(iGEM)是每年其中一個最大型的國際科研比賽。每年，參加隊伍會進行一研究，而他們會在年底於美國波士頓的Giant　Jamboree上向其他隊伍以及專業評審團隊匯報研究成果，以競逐如最佳研究，最佳報告等各個獎項。

除了研究方面，iGEM隊伍更需向公眾宣傳合成生物學。他們會用盡各種方法，如公眾展覽，實驗展示，訪問等來達到目的。他們希望透過這些方法引起公眾對合成生物學的認知，以及令公眾了解合成生物學潛在的議題。

這正是我們現在進行的。

Table of Contents
目錄

頁數 Page

What is DNA
DNA是甚麼 1

MicroRNAs: important functions in the body
微核醣核酸：體內的重要用途 2

Strand displacement
鏈置換原理 3

G-quadruplex: formation and utilization
鳥嘌呤四螺旋體：生成與應用 4

Molecular beacon: FRET-based signal production
分子信標：透過螢光共振能量轉移產生訊號 5

Synthetic biology
合成生物學
- Introduction
  簡介 6
- Applications
  應用 7

What is DNA
DNA是甚麼

DNA is the fundamental unit of all lives. It stores all information of a specific organism. DNA is naturally found in the nucleus of cells.

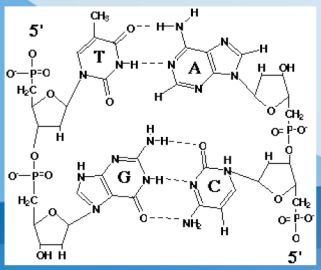
DNA comprises of four bases: adenine (A), thymine (T), cytosine (C) and guanine (G). A pairs with T and C pairs with G through hydrogen bonds. All of these combine to form a double helix structure.

Nowadays, DNA can be synthesized in vitro, which has aroused many interests towards its vast applications.

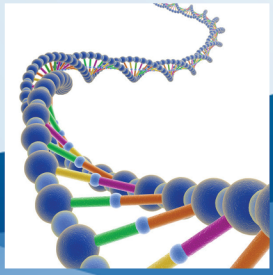
DNA(去氧核糖核酸)是所有生命的最基本元素。它平常出現在細胞核裡，並儲存著特定生物的所有資訊。

DNA由四個鹼基組成，它們分別是腺嘌呤(A)，胸腺嘧啶(T)，鳥嘌呤(G)和胞嘧啶(C)。A必定與T組合，C也必定與G組合，所形成的便是一個雙螺旋結構。

現今，DNA已能夠在體外合成，這種技術亦帶來很多廣泛的應用。



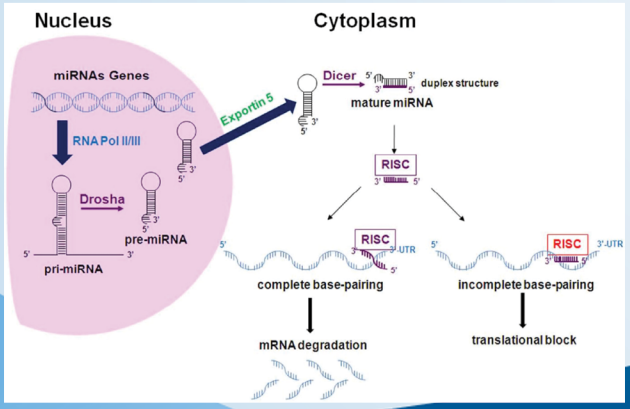
The four bases of DNA and their pairings.
DNA的四個鹼基與它們的組合。


DNA double helix structure.
DNA雙螺旋結構。

MicroRNAs: important functions in the body
微核醣核酸：體內的重要用途

MicroRNAs (miRNAs), usually of around 22 nucleotides long, are made inside our body via complex mechanisms. They play important roles in gene regulation through several ways, such as binding with mRNA to inhibit translation and speeding up mRNA degradation to cause gene silencing. Dysregulation of miRNA expression may lead to under- or over-expression of genes and hence diseases.

微核醣核酸(miRNA)，長度約二十二核苷酸，是人體內透過複雜方法而製作的。它們在控制基因表現方面扮演重大的角色，例如與信使核醣核酸(mRNA)結合，提升mRNA的分解速度從而導致基因靜默。miRNA失調可導致過低或過高的基因表現，從而產生疾病。

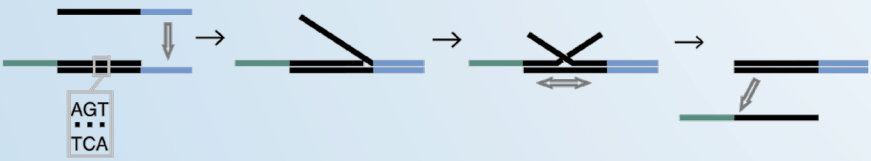


The process of miRNA synthesis.
miRNA的合成方法。

Strand displacement
鏈置換原理

Strand displacement reaction is commonly employed in DNA nanostructure designs. During the reaction, two strands with partly or fully complementary sequences hybridize to each other, displacing one or more pre-hybridized strands. This process is initiated at a single-stranded site called a 'toehold'.

在DNA納米結構設計上，很多時候都會使用鏈置換反應。在反應期間，兩條部分或完全配合的基因排序會相互雜交，從而把一條或多條本身配合的基因鏈置換掉。這反應在名叫toehold的單鏈位置開始。

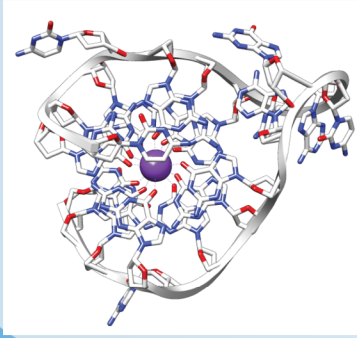


A clear picture showing the mechanism of strand displacement. Try to find the toehold here.
圖解鏈置換原理。Toehold在上圖中的哪個位置？

G-quadruplex: formation and utilization
鳥嘌呤四螺旋體：生成與應用

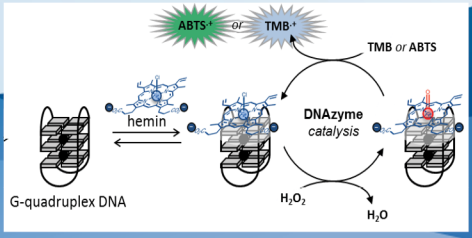
G-quadruplex (Gq) is formed by 4 strands of DNA made up of guanine bases. When Gq forms a complex with hemin, it exhibits peroxidase activity and functions as a DNAzyme. Its catalytic activity is utilized in many DNA nanostructures where a colour change is produced by target-induced conformational change.

鳥嘌呤四螺旋體(Gq)是由四條很高鳥嘌呤鹼基含量的DNA鏈組成。當Gq與氯化血紅素合成一體時，便會展現出過氧化物酶活動，從而作為一個DNA酶。它的催化活動可被運用於不少DNA納米結構，透過目標誘發的結構轉變而改變顏色。



A 3D representation of basic G-quadruplexes.
Gq的基本三維結構。

Basic ABTS or TMB assay. Hemin binds to G-quadruplex which in turn gives peroxidase activity, turning ABTS from light green to a deeper green colour or turning TMB from colourless to blue.
基本的ABTS(2,2-聯氮-二(3-乙基-苯並噻唑-6-磺酸)二銨鹽)或TMB測定方法。Gq與氯化血紅素合成一體，產生過氧化酶活動，把ABTS和TMB分別變成深綠色以及藍色。

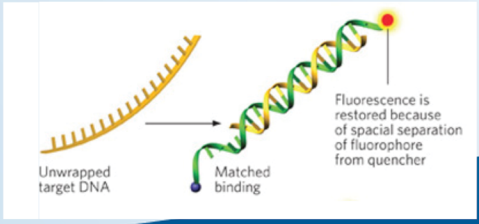


Molecular beacon: FRET-based signal production
分子信標：透過螢光共振能量轉移產生訊號

Some DNA nanostructures make use of fluorophores and quenchers, which are often termed as a 'Molecular Beacon'. The quencher acts as a suppressor which inhibits fluorescence of the fluorophore when the two are in close proximity. When the two are separated by target-induced conformational change, the suppressing effect is cancelled, resulting in the restoration of fluorescence. The higher the concentration of targets in a sample, the higher the absorbance. Therefore, DNA nanostructures with molecular beacons (having specific miRNAs as targets) can be used for measuring miRNA expression level, which may reveal clinical states.

有些其他的DNA納米結構亦會運用到螢光團與抑制器，它們通常被稱為分子信標。當螢光團很靠近抑制器時，抑制器便會把螢光團的螢光抑制。當出現目標誘發的結構轉變時，抑制效果便會消除，從而恢復螢光。目標在樣品中的濃度越高，吸光度亦會相應地提高。因此，透過特定miRNA作為目標分子，DNA納米結構配合分子信標便能被用作檢測miRNA表現的程度，從而揭示病情。

Structure of a basic molecular beacon.
基本分子信標的結構。

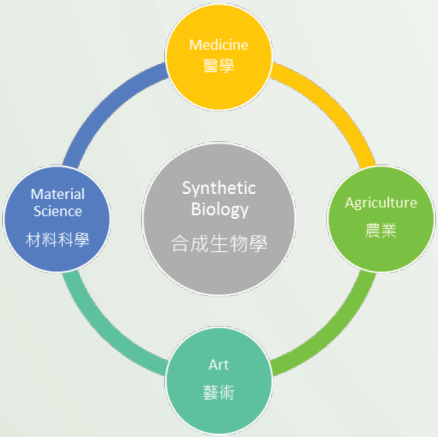


FRET principle. Refer to our lego model.
螢光共振能量轉移原理。還記得我們製作的樂高模型嗎？

Synthetic biology
合成生物學

Synthetic biology is a discipline that uses engineering principles to design and assemble biological components. It can be applied in many fields, including medicine, material science and agriculture. Synthetic biology is enabled by many technologies, such as DNA synthesis and editing, computer modelling and large-scale bacteria culturing.

合成生物學是一門利用工程學原理來設計和組成生物組件的學科。它在各領域有不同的應用，並且是在各種先進技術，如DNA合成與改造，電腦塑造法與大型細菌培養等的發展下應運而生。



Synthetic biology applications.
合成生物學的應用。

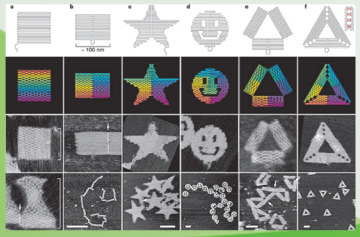
Synthetic biology applications: material science
應用層面：材料科學

The use of DNA can be extended beyond genetic encoding. For example, DNA origami was built using complementary base-pairing of DNA. The first utilization of this resulted in a smiley face which received unprecedented recognition and sparked endless enthusiasm on related fields. New origami are being built constantly: boxes, flags, stars, dolphins... and whatever one can dream of.

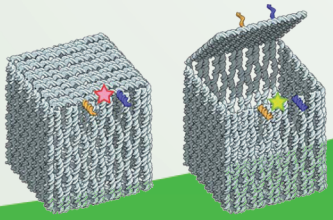
Apart from an art, DNA origami can also be drug carriers when they are in the form of boxes, or materials for information storage if DNA strands are knitted into maps.

DNA除了儲存資訊外，亦能有其他用途。在藝術方面，我們可利用DNA的鹼基配對原理製作DNA摺紙，在顯微鏡下可顯示出不同形狀，例如最初被設計出來的笑臉，國旗等。利用這種技術，新的形狀亦隨之面世，例如星形，海豚等。

在應用層面，DNA摺紙也可以被製成盒狀的藥物載體，或地圖狀的資訊儲存物料。



DNA origami as artistic nanopieces.
DNA摺紙被製作成納米藝術作品。

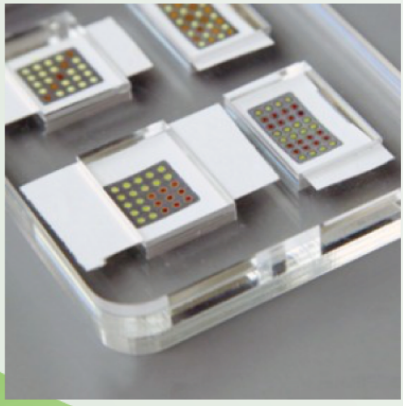


DNA origami as boxes for drug carriers.
DNA摺紙被製作成盒狀的藥物載體。

Synthetic biology applications: disease diagnosis
應用層面：疾病檢測

Commercially available synthetic bacterial transcription-translation systems can be freeze-dried onto paper or other porous materials. These specially designed systems make proteins only if they're triggered by the target DNA or RNA. They can be activated when rehydrated, allowing the freeze-dried material to be easily transported. Recent invention of an Ebola virus RNA detection system using color-changing enzymes is an excellent example.

市場上可買到的細菌DNA轉錄-轉譯系統可以在紙或其他具穿透力的物質上冷乾。這些系統在檢測到特定的DNA或RNA時才會誘發一連串反應，製造出帶顏色的蛋白質。被冷乾的系統在再水化後便會被激活，使這些系統能很容易地被運送。最近發明的伊波拉病毒RNA檢測系統就是用了上述方法 。

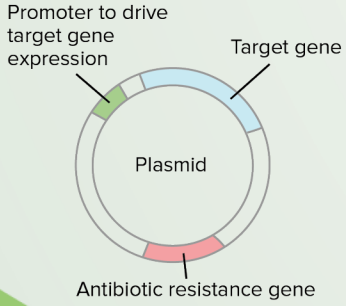


Paper-based diagnostic for Ebola. Enzymes present on the diagnostic paper will change colour when Ebola viral RNA is detected.
伊波拉病毒RNA檢測系統。當伊波拉病毒RNA出現在試紙上，試紙的顏色便會改變。

Synthetic biology applications: Production lines
應用層面：微型生產線

Bacteria such as *E. coli* can be turned into factories via transformation. Recombinant plasmids with the gene coding for the desired products (e.g. ssDNA, enzymes required) are cloned into the bacteria. The useful products (e.g. ssDNA) are extracted, purified, and further processed, ready for producing a great variety of molecules, from human hormones to food flavorings.

細菌如大腸桿菌可透過轉型成為微型工廠。我們可以把想要的基因放進質粒，並把它們送進細菌。只要把細菌培養得好，它們便能生產大量同樣的基因以及該基因所表現的東西，如人類荷爾蒙，調味料等。



A trandformed plasmid structure.
已被重組的質粒結構。

References
1. Medgadget. Smart Nanobox Built Out of DNA Origami. Retrieved 17.8.2016 from http://www.medgadget.com/2009/05/smart_nanobox_built_out_of_dna_origami.html
2. Rothemund, P. W. (2006). Folding DNA to create nanoscale shapes and patterns. Nature, 440(7082), 297-302.
3. MIT Technology Review (2014). Synthetic Biologists Create Paper-Based Diagnostic for Ebola.
4. U.S. Department of Agriculture (2015). Detection test for subclinical mastitis in dairy cows developed.
5. Rota R., Ciarapica R., Giordano A., Miele L. & Locatelli F. (2011). MicroRNAs in rhabdomyosarcoma: Pathogenetic implications and translational potentiality. Molecular Cancer 10(1):120.
6. Machinek, R. R., Ouldridge, T. E., Haley, N. E., Bath, J., & Turberfield, A. J. (2014). Programmable energy landscapes for kinetic control of DNA strand displacement. Nature communications, 5.
7. Zielinska E (2012). A Brighter Beacon: A novel liquid laser set-up can detect single nucleotide mutations in a cancer gene.
8. Loic, S. (2016). Native and Synthetic G-quartet-based DNAzyme Systems–Artificial Enzymes for Biotechnological Applications. world, 1, 6.

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